Claims

1. A regulatory site for a mitochondrial proton leak wherein the site is activated by adenosine monophosphate (AMP).

2. The use of a regulatory site according to claim 1 in a screening assay to identify compounds which are useful in the treatment of a body weight disorder such as obesity or cachexia or related co-morbid conditions.

3. A screening method for the identification of compounds which modulate an AMP-sensitive regulatory site on mitochondria comprising the steps of:
   a) contacting a test compound with mitochondria in the presence of a substrate for respiration in the presence of a buffer system;
   b) measuring an index of metabolic rate; and
   c) identifying compounds which modulate the metabolic rate.

4. A method according to claim 3 which further comprises the steps of
   a) contacting the compounds identified in claim 3 with mitochondria in the presence of a substrate for respiration in the presence of a buffer system and in the presence of AMP and measuring an index of metabolic rate; and
   b) comparing the metabolic rate in claim 3 step (b) and claim 4 step (a) and identifying compounds where there is not an additive effect on metabolic rate as compounds which modulate the AMP-sensitive regulatory site.

5. A method according to claim 3 or 4 wherein in step c) compounds which increase the metabolic rate are identified as compounds which activate an AMP-sensitive regulatory site on mitochondria.

6. A method according to claim 3 or 4 wherein in step c) compounds which decrease the metabolic rate are identified as compounds which inhibit an AMP-sensitive regulatory site on mitochondria.

7. A screening method for the identification of compounds which modulate an AMP-sensitive regulatory site on mitochondria comprising the steps of:
   a) contacting a test compound with mitochondria in the presence of a substrate for respiration in the presence of a buffer system;

b) measuring the membrane potential; and

c) identifying compounds which change the membrane potential.

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8. A method according to claim 7 which further comprises the steps of:

a) contacting the compounds identified in claim 7 with mitochondria in the presence of a substrate for respiration in the presence of a buffer system and in the presence of AMP and measuring membrane potential, and

b) comparing the membrane potential in claim 7 step (b) and claim 8 step (a) and identifying compounds where there is not an additive effect on membrane potential as compounds which modulate the AMP-sensitive regulatory site.

9. A method according to claim 7 or 8 wherein in step c) compounds which decrease the membrane potential are identified as compounds which activate an AMP-sensitive regulatory site on mitochondria.

10. A method according to claim 7 or 8 wherein in step c) compounds which increase the membrane potential are identified as compounds which inhibit an AMP-sensitive regulatory site on mitochondria.

11. A screening method for the identification of compounds which modulate an AMP-sensitive regulatory site on mitochondria comprising the steps of:

a) contacting a test compound with mitochondria in the presence of a substrate for respiration in the presence of a buffer system;

b) measuring an index of metabolic rate and measuring the membrane potential; and

c) identifying compounds which change the metabolic rate and change the membrane potential as compounds which modulate the AMP-sensitive regulatory site.

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12. A method according to claim 11 which further comprises the steps of

a) contacting the compounds identified in claim 11 with mitochondria in the presence of a substrate for respiration in the presence of a buffer system and in the presence of AMP, measuring an index of metabolic rate and measuring the membrane potential; and

b) comparing metabolic rate and the membrane potential in claim 11 step (b) and claim 12 step (a) and identifying compounds where there is not an additive effect on metabolic rate and membrane potential as compounds which modulate the AMP-sensitive regulatory site.

13. A method according to claim 11 or 12 wherein in step c) compounds which increase the metabolic rate and decrease the membrane potential are identified as compounds which activate an AMP-sensitive regulatory site on mitochondria.

14. A method according to claim 11 or 12 wherein in step c) compounds which decrease the metabolic rate and increase the membrane potential are identified as compounds which inhibit an AMP-sensitive regulatory site on mitochondria.

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15. A method according to any one of claims 3 to 6 and 11 to 14 wherein the index of metabolic rate is oxygen consumption.

16. A method according to any one of claims 3 to 15 wherein the mitochondria are isolated mitochondria or a suitable part or derivative thereof.

17. A method according to any one of claims 3 to 15 wherein the mitochondria are skeletal muscle mitochondria or a suitable part or derivative thereof.

18. A method according to claim 17 wherein the skeletal muscle mitochondria are rat skeletal muscle mitochondria.

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19. A method according to any one of claims 3 to 15 wherein the mitochondria are present in intact eukaryotic cells.

20. A method according to claim 19 wherein the intact cells present in tissue slices of mammalian origin or cell lines of mammalian origin.

Sub A6

21. A method according to any one of claims 3 to 15 wherein a complex 1 inhibitor is present.

Sub A6

22. A method according to any one of claims 3 to 15 wherein the substrate is a succinate salt.

23. A method according to claim 21 wherein complex 1 inhibitor is rotenone.

Sub A7

24. A method according to any one of claims 3 to 22 wherein the screening method is carried out in the presence of varying concentrations of an electron transport inhibitor.

25. A method according to claim 24 wherein the electron transport inhibitor is selected from a malonate salt, myxothiazol or a cyanide salt.

26. A method according to claim 24 wherein the electron transport inhibitor is a malonate salt.

27. A screening method according to claim 15 wherein the oxygen consumption is measured by an oxygen electrode.

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28. A screening method according to any one of claims 9 to 26 wherein the membrane potential is measured using ion selective electrodes.

29. A screening method according to any one of claims 9 to 26 wherein the membrane potential is measured using fluorescent membrane potential dyes.

30. A screening method according to any previous claim wherein an inhibitor of ATP synthesis is present.

31. A method according to any one of claims 3 to 30 for the identification of compounds which are suitable for use in the treatment of a body weight disorder.

32. A method according to any one of claims 5, 9, and 13 for the identification of compounds which are suitable for use in the treatment of obesity and related conditions.

Sub A8

33. A method according to any one of claims 6, 10 and 14 for the identification of compounds which are suitable for use in the treatment of cachexia and related conditions.

34. A screening method for the identification of compounds which are useful in the treatment of a body weight disorder the method comprising the step of identifying a compound which selectively binds to ANC.

35. A screening method for the identification of compounds which are useful in the treatment of obesity and related conditions the method comprising the step of identifying an agonist of ANC.

36. A screening method for the identification of compounds which are useful in the treatment of cachexia and related conditions the method comprising the step of identifying an antagonist of ANC.

37. A binding assay for the identification of compounds which are suitable for use in the treatment of obesity and related conditions comprising the steps of:

a) incubating an ANC-containing preparation with a labelled ligand to produce a labelled ANC-containing preparation;

b) contacting a test compound with the labelled ANC-containing preparation; and

c) identifying a compound which reduces the amount of labelled ligand present in the ANC-containing preparation as a compound which may suitable for use in the treatment of obesity.

38. An assay according to claim 37 in which the ANC-containing preparation comprises one of the following: a) intact tissue preparations b) cell lines, from a skeletal muscle source or from a cardiac source or from an aortic smooth muscle source c) cells into which ANC has been introduced by genetic means d) cells isolated from tissues for example from cardiac or skeletal muscle tissues e) membranes ; f) mitochondria ; g) mitochondrial membranes or h) isolated ANC preferably in purified form.

39. An assay according to either claim 37 or claim 38 in which the ANC-containing preparation is a cell line which is optionally upregulated.

Sub A9

40. An assay method according to any one of claims 37 to 39 in which the labelled ligand is radiolabelled or fluorescently labelled atractylate or fluorescently labelled ATP or ADP.

41. A screening method for the identification of compounds which are useful in the treatment of a body weight disorder the method comprising the step of identifying a compound which modulates the mitochondrial proton leak mediated by an ANC.

42. A screening method for identification of compounds which are useful in the treatment of obesity and related conditions the method comprising the step of identifying a compound which enhances mitochondrial proton leak mediated by an ANC.

43. A screening method for identification of compounds which are useful in the treatment of cachexia and related conditions the method comprising the step of identifying a compound which reduces mitochondrial proton leak mediated by an ANC.

Sub A10

44. A (functional) screening method for identifying compounds which modulate the proton leak mediated by an ANC comprising the steps of :

a) incubating a test compound with cells in which an ANC is upregulated and measuring an index of metabolic rate or membrane potential

b) incubating a test compound with control cells in which the ANC used in step a) is absent or is present at lower levels than in step a) and measuring an index of metabolic rate or membrane potential and

c) identifying a compound which gives rise to a different metabolic rate or different membrane potential in step a) compared to step b) as a compound which modulates the proton leak mediated by an ANC.

45. A method according to claim 44 wherein in step c) a compound which gives rise to an increased metabolic rate or decreased membrane potential is identified as a compound which enhances the proton leak mediated by an ANC.

46. A method according to claim 44 wherein in step c) a compound which gives rise to a decreased metabolic rate or increased membrane potential is identified as a compound which reduces proton leak mediated by an ANC.

47. A method according to any one of claims 44 to 46 for the identification of compounds which are suitable for use in the treatment of a body weight disorder.

48. A method according to claim 45 for the identification of compounds whichare suitable for use in the treatment of obesity and related conditions.

49. A method according to claim 46 for the identification of compounds whichare suitable for use in the treatment of cachexia and related conditions.

50. A method or assay according to any one of claims 3 to 49 further comprising the step of screening a compound identified as being suitable for use in the treatment of a body weight disorder in a further screen for suitability in treating a body weight disorder.

SubA 11

51. A method or assay according to any one of claims 5, 9, 13, 35, 42, 45 and 48 further comprising the step of screening a compound identified in a further screen for suitability in treating obesity or a related condition.

52. A method or assay according to any one of claims 6, 10, 14, 36, 43, 46 and 49 further comprising the step of screening a compound identified in a further screen for suitability in treating cachexia or a related condition.

53. A compound identifiable in a screening method or assay according to any one of claims 3 to 52.

54. A compound identified in a screening method or assay according to any one of claims 3 to 52.

55. A compound according to claim 53 or 54 for use in medicine.

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56. A method for treating a body weight disorder in a patient the method comprising administering to the patient a compound according to claim 53 or 54.

57. Use of a compound according to claim 53 or 54 in the manufacture of a medicament for treating a body weight disorder.

58. A method of treating a patient with obesity or a related co-morbid condition the method comprising administering to the patient an agonist of an AMP-sensitive regulatory site on mitochondria or an agonist of the AMP effect on the ANC mediated proton leak.

59. Use of an agonist of an AMP-sensitive regulatory site on mitochondria or an agonist of the AMP effect on the ANC mediated proton leak in the manufacture of a medicament for treating obesity or a related co-morbid condition.

60. A method according to claim 58 or the use according to claim 59 wherein the activator of an AMP-sensitive regulatory site on mitochondria is any one of6-chloropurineriboside 5'-monophosphate, cordecypin 5'-monophosphate and xanthosine 5'-monophosphate.